



Pending Claims  
U.S.S.N. 09/523,809  
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1. A cultured tissue construct comprising fibroblasts cells grown under conditions to produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

- (i) fibrillar collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;
- (ii) decorin; and,
- (iii) glycosaminoglycans;

and wherein said extracellular matrix is produced by the cultured fibroblast cells in the absence of exogenous matrix components or synthetic members during the culturing conditions.

2. The cultured tissue construct of claim 1, wherein fibroblasts are derived from tissue selected from the group consisting of neonate male foreskin, dermis, tendon, lung, urethra, umbilical cord, corneal stroma, oral mucosa, and intestine.

3. The cultured tissue construct of claim 1, wherein said cultured cells are dermal fibroblasts.

4. The cultured tissue construct of claim 1, wherein said cultured cells are from dermal papilla of hair follicles.

5. The cultured tissue construct of claim 1, wherein said layer has cultured cells from dermal papilla of hair follicles are localized on said layer.

6. The cultured tissue construct of claim 1, wherein said cultured cells are cultured in chemically defined media.

7. The cultured tissue construct of claim 1, wherein said cultured cells are derived from human tissue and are cultured in medium containing no non-human components.

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8. A cultured tissue construct comprising cultured dermal fibroblasts cells cultured under conditions to produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

- (i) fibrillar type I and type III collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;
- (ii) decorin,
- (iii) fibronectin
- (iv) tenascin; and,
- (v) glycosaminoglycans;

and wherein said extracellular matrix is produced by the cultured fibroblast cells in the absence of exogenous matrix components or synthetic members during the culturing conditions.

9. A cultured tissue construct having at least two layers, comprising:

(a) a first layer of cultured fibroblasts cells cultured under conditions to produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

- (i) fibrillar collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;
- (ii) decorin; and,
- (iii) glycosaminoglycans;

wherein said extracellular matrix is produced by the cultured fibroblast cells in the absence of exogenous matrix components or synthetic members during the culturing conditions; and,

(b) a second layer of cells comprising epithelial cells disposed on the first layer.

10. The bilayered cultured tissue construct of claim 9, wherein the epithelial cells are selected from the group consisting of keratinocytes, corneal epithelial cells, epithelial cells from oral mucosa, esophageal epithelial cells, and uroepithelial cells.

11. The bilayered cultured tissue construct of claim 9, wherein said fibroblast cells contained within said first layer are derived from tissue selected from the group consisting of neonate male foreskin, dermis, tendon, lung, cartilage, urethra, corneal stroma, oral mucosa, and intestine.

12. The bilayered cultured tissue construct of claim 9, wherein said fibroblast cells contained within said first layer are dermal fibroblasts.

13. The bilayered cultured tissue construct of claim 12, wherein said fibroblast cells contained within said first layer are from dermal papilla of hair follicles.

14. The bilayered cultured tissue construct of claim 9, wherein said first layer has cultured cells from dermal papilla of hair follicles are localized on said first layer.

15. The bilayered cultured tissue construct of claim 9, further comprising a third layer of cells disposed on the second layer of epithelial cells.

16. A cultured skin construct having at least two layers, comprising:

(a) a first layer of cultured dermal fibroblasts cells cultured under conditions to produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

(i) type I and type III collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;

(ii) decorin;

(iii) fibronectin,

(iv) tenascin; and,

(v) glycosaminoglycans;

wherein said extracellular matrix is produced by the cultured dermal fibroblast cells in the absence of exogenous matrix components or synthetic members during the culturing conditions; and,

(b) a second layer of keratinocyte cells disposed on the first layer to form an epidermal cell layer, wherein the epidermal cell layer is a multilayered, stratified, differentiated and exhibits a basal layer, suprabasal layer, a granular layer and a stratum corneum;

and wherein the bilayered cultured skin construct has a basement membrane present at the junction of the first and second layers.

17. The construct of any of claims 1, 8, 9, and 16, wherein the cultured fibroblast cells are genetically modified to produce extracellular matrix components.

18. The construct of claim 17, wherein the cultured fibroblast cells are genetically modified to produce a growth factor, hormone, peptide, or protein.

19. (Amended) A method for producing a cultured tissue construct, comprising,

(a) seeding fibroblast cells capable of synthesizing an extracellular matrix on a porous membrane in a culture vessel in a first cell culture medium;

(b) culturing the fibroblast cells of step (a) in the first cell culture medium to between about 80% to about 100% confluence on the porous membrane;

(c) stimulating the fibroblast cells to synthesize, secrete and organize extracellular matrix components by culturing the cells in a second culture medium; and,

(d) continued culturing of the fibroblast cells until the cells form a layer of synthesized extracellular matrix of at least about 30 microns thick, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

(i) fibrillar collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;

(ii) decorin; and,

(iii) glycosaminoglycans;

and wherein said extracellular matrix is produced by the cultured fibroblast cells in the absence of exogenous matrix components or synthetic members during the culturing conditions.

20. The method of claim 19, wherein either the first culture medium, or the second culture medium, or both the first and second culture mediums are chemically defined.

21. The method of claim 19, wherein the first and second culture mediums contain no non-human components.

22. The method of claim 19, wherein in step (a) the fibroblast cells are seeded at a density between about  $1 \times 10^5$  cells/cm<sup>2</sup> to about  $6.6 \times 10^5$  cells/cm<sup>2</sup>.

23. The method of claim 19, wherein the fibroblast cells are derived from tissue selected from the group consisting of neonate male foreskin, umbilical cord, dermis, tendon, lung, urethra, corneal stroma, oral mucosa, and intestine.

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24. (Amended) A method for producing a bilayered cultured tissue construct, comprising:

(a) seeding fibroblast cells capable of synthesizing an extracellular matrix on a porous membrane in a culture vessel in a first cell culture medium;

(b) culturing the fibroblast cells of step (a) in the first cell culture medium to between about 80% to about 100% confluence on the porous membrane;

B2 (c) stimulating the fibroblast cells of step (a) to synthesize, secrete and organize extracellular matrix components by culturing the cells in a second culture medium;

(d) continued culturing of the fibroblast cells until the cells form a layer of synthesized extracellular matrix of between about 30 to about 110 microns thick, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

(i) fibrillar collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;

(ii) decorin; and,

(iii) glycosaminoglycans;

and wherein said extracellular matrix is produced by the cultured fibroblast cells in the absence of exogenous matrix components or synthetic members during the culturing conditions;

(e) seeding epithelial cells to the top surface the synthesized extracellular matrix of step (d), and,

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(f) stimulating the epithelial cells of step (e) under culturing conditions to form a bilayered tissue construct of an extracellular matrix, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, and a second layer of epithelial cells.

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25. The method of claim 24, wherein said fibroblast cells capable of synthesizing an extracellular matrix are derived from tissue selected from the group consisting of neonate male foreskin, dermis, tendon, lung, cartilage, urethra, corneal stroma, oral mucosa, and intestine.

26. The method of claim 24, wherein the epithelial cells are selected from the group consisting of keratinocytes, corneal epithelial cells, epithelial cells from oral mucosa, esophageal epithelial cells, and uroepithelial cells.

27. A method for producing a bilayered cultured skin construct comprising a dermal layer and an epidermal layer disposed thereon in the absence of a structural support scaffold or exogenous matrix components, wherein the method comprises the steps of:

(a) seeding fibroblast cells on porous membrane in a culture vessel in a first chemically defined cell culture medium at a density between about  $1 \times 10^5$  cells/cm<sup>2</sup> to about  $6.6 \times 10^5$  cells/cm<sup>2</sup>;

(b) culturing the fibroblast cells of step (a) in the first chemically defined cell culture medium to between about 80% to about 100% confluence;

(c) stimulating the fibroblast cells of step (a) to synthesize, secrete and organize extracellular matrix components by culturing the cells in a second chemically defined culture medium;

(d) continued culturing of the fibroblast cells until the cells form a layer of synthesized extracellular matrix of at least about 30 microns thick, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer,

wherein the extracellular matrix comprises:

(i) type I and type III collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;

(ii) decorin;

(iii) tenascin; and,

(iv) glycosaminoglycans;

wherein said extracellular matrix is produced by the cultured dermal fibroblast cells in the absence of exogenous matrix components or synthetic members during the culturing conditions;

(e) seeding keratinocyte cells to the top surface of the synthesized extracellular matrix of step (d), and,

(f) culturing the keratinocyte cells under culturing conditions to form an epidermal layer,

wherein the epidermal cell layer is a multilayered, stratified, differentiated layer of keratinocytes that exhibit a basal layer, a suprabasal layer, a granular layer and a stratum corneum;

and wherein the bilayered cultured skin construct has a basement membrane present at the junction of the first and second layers.

28. A method for transplantation or implantation of a cultured tissue construct into a patient comprising transplanting or implanting a cultured tissue construct of any of claims 1, 8, 9 or 16 into a patient in need of treatment thereof.

29. A method for producing a cultured tissue construct, comprising,

(a) seeding fibroblast cells capable of synthesizing an extracellular matrix on a porous membrane in a culture vessel in a cell culture medium at about 80% to about 100% confluence;

(b) stimulating the fibroblast cells to synthesize, secrete and organize extracellular matrix components under culturing conditions in a second culture medium; and,

(c) continued culturing of the fibroblast cells until the cells form a layer of synthesized extracellular matrix of at least about 30 microns thick, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

(i) fibrillar collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;

(ii) tenascin; and,

(iii) glycosaminoglycans;

and wherein said extracellular matrix is produced by the cultured fibroblast cells in the absence of exogenous matrix components or synthetic members during the culturing conditions.

30. The construct of any of claims 1-18, wherein the construct is cohesive in having physical unitary integrity and tissue-like handling properties.